

teams are key mechanisms for helping public health authorities manage the COVID-19 crisis. Our findings revealed that the P.1 VOC was introduced into Uruguay multiple times over a period of increasing mobility in binational cities along the Brazil–Uruguay border and in Uruguay between mid-February and early March 2021. The introduction of the highly transmissible P.1 VOC coupled with the increasing human mobility probably contributed to the rapid local spread of this variant and the worsening COVID-19 epidemic in Uruguay during January–July 2021.

This article was preprinted at <https://www.medrxiv.org/content/10.1101/2021.05.20.21256969v1>.

### Acknowledgments

We thank the groups that contributed SARS-CoV-2 genomes to the EpiCoV GISAID initiative (Appendix 2). We thank Marcelo Fiori and María Inés Fariello for sharing mobility data from Uruguay and Christian Brandt from Institute for Infectious Diseases and Infection Control (Jena University Hospital), for adapting their poreCov Nextflow pipeline to our requirements and for clarifying doubts about its implementation. We thank Nicolas Nin and Javier Hurtado from Hospital Español for their work as medical doctors caring for patients included in this study.

This work was supported by FOCEM-Fondo para la Convergencia Estructural del Mercosur (COF03/11).

### About the Author

Ms. Rego is a research biologist and trained in bioinformatics at Institut Pasteur de Montevideo, Uruguay. Her research interests include evolutionary biology, transcriptomics, host-pathogen interactions, and the epidemiology and phylogeny of coronaviruses.

### References

- Abdool Karim SS, de Oliveira T. New SARS-CoV-2 variants—clinical, public health, and vaccine implications. *N Engl J Med.* 2021;384:1866–8. <https://doi.org/10.1056/NEJMc2100362>
- Centers for Disease Control and Prevention. SARS-CoV-2 variant classifications and definitions [cited 2021 Apr 10]. <https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html>
- Faria NR, Mellan TA, Whittaker C, Claro IM, Cândido DDS, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science.* 2021;372:815–21. <https://doi.org/10.1126/science.abb2644>
- Fiocruz Genomic Network. Frequency of the main SARS-CoV-2 lineages per month sampling [in Portuguese] [cited 2021 Apr 30]. <http://www.genomahcov.fiocruz.br/frequencia-das-principais-linhagens-do-sars-cov-2-por-mes-de-amostragem>
- Outbreak.info. P.1 lineage report [cited 2021 Apr 30]. [https://outbreak.info/situation-reports?pango=P.1&selected=BRA&loc=BRA&loc=USA&loc=USA\\_US-CA](https://outbreak.info/situation-reports?pango=P.1&selected=BRA&loc=BRA&loc=USA&loc=USA_US-CA)
- Our World in Data. Daily new and confirmed COVID-19 cases per million people [cited 2021 Jun 30]. <https://ourworldindata.org/explorers/coronavirus-data-explorer>
- Elizondo V, Harkins GW, Mabvakure B, Smidt S, Zappile P, Marier C, et al. SARS-CoV-2 genomic characterization and clinical manifestation of the COVID-19 outbreak in Uruguay. *Emerg Microbes Infect.* 2021;10:51–65. <https://doi.org/10.1080/2221751.2020.1863747>
- Mir D, Rego N, Resende PC, Tort F, Fernández-Calero T, Noya V, et al. Recurrent dissemination of SARS-CoV-2 through the Uruguayan–Brazilian border. *Front Microbiol.* 2021;12:653986. <https://doi.org/10.3389/fmicb.2021.653986>
- Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol.* 2020;5:1403–7. <https://doi.org/10.1038/s41564-020-0770-5>

Address for correspondence: Pilar Moreno, Laboratorio de Virología Molecular, Facultad de Ciencias, Universidad de la República, Mataojo 2055, Montevideo, Uruguay; email: pmoreno@pasteur.edu.uy

## Highly Pathogenic Avian Influenza A(H5N1) Virus in Wild Red Foxes, the Netherlands, 2021

Jolianne M. Rijks, Hanna Hesselink, Pim Lollinga, Renee Wesselman, Pier Prins, Eefke Weesendorp, Marc Engelsma, Rene Heutink, Frank Harders, Marja Kik, Harry Rozendaal, Hans van den Kerkhof, Nancy Beerens

Author affiliations: Dutch Wildlife Health Centre, Utrecht University, Utrecht, the Netherlands (J.M. Rijks, H. Hesselink, M. Kik); Stichting Faunavisie Wildlife Care, Westernieland, the Netherlands (P. Lollinga); Stichting Faunavisie Wildlife Care, Blijham, the Netherlands (R. Wesselman); Dierenkliniek Winsum, Winsum, the Netherlands (P. Prins); Wageningen Bioveterinary Research, Lelystad, the Netherlands (E. Weesendorp, M. Engelsma, R. Heutink, F. Harders, N. Beerens); Dutch Food and Consumer Products Safety Authority, Utrecht (H. Rozendaal); Coordination Centre for Communicable Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands (H. van den Kerkhof)

DOI: <https://doi.org/10.3201/eid2711.211281>

We detected infection with highly pathogenic avian influenza A(H5N1) virus clade 2.3.4.4b in 2 red fox (*Vulpes vulpes*) cubs found in the wild with neurologic signs in the Netherlands. The virus is related to avian influenza viruses found in wild birds in the same area.

**O**n May 10, a red fox *Vulpes vulpes* cub (cub 1) displaying abnormal behavior was found in Bellingwolde, the Netherlands, and taken into care of a wildlife rescue center. Upon entry, the 6- to 8-week-old cub was slightly dehydrated and showed at intervals of <30 minutes lip retraction, rapid opening and closing of mouth, excessive salivation, skin twitching, head shaking, and body tremors (Figure; Video, <https://wwwnc.cdc.gov/EID/article/27/11/21-1281-V1.htm>). The cub first seemed to improve, but on May 12 it reacted aggressively when touched. Subsequently, we observed difficult swallowing and labored breathing. The cub seemed blind and stopped eating. As the situation further deteriorated, we humanely euthanized the cub on May 16. On May 13, the center received another 6- to 8-week-old red fox cub (cub 2) found ≈900 m from cub 1. Cub 2 was hypothermic and dehydrated. It had seizures and died overnight.

Retrospectively, we concluded that the mother of the cubs was likely a vixen found walking circles on May 10, ≈975 m direct distance from cub 1 and ≈90 m from cub 2. The vixen reacted very aggressively to capture, responding to sound but blind. The vixen had a fresh elbow fracture, probably caused by a road traffic accident. We humanely euthanized her the same day and sent her carcass for destruction.

Although rabies lyssavirus is unlikely in the Netherlands, European bat 1 lyssavirus is endemic in serotine bats (*Eptesicus serotinus*) (1). To exclude lyssavirus infection in the fox cubs, we performed a direct fluorescent antibody test on smears of brain tissue in accordance with World Organisation for Animal Health (OIE) protocol ([standards/tahm/3.01.17\\_RABIES.pdf\). Test results were negative.](https://www.oie.int/fileadmin/Home/eng/Health_</a></p>
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Subsequently, we tested brain samples for avian influenza virus by using a PCR detecting the influenza A virus matrix gene, followed by the subtype-specific H5-PCR on the hemagglutinin gene, as described previously (2). The samples from both cubs tested positive (Table), and we subtyped the virus as highly pathogenic avian influenza (HPAI) influenza virus A subtype H5N1. We isolated the HPAI H5N1 virus from the brain of cub 1 by inoculation of the samples into 10-day-old embryonated special pathogen-free chicken eggs.

During April–May 2021, large numbers of dead barnacle geese (*Branta leucopsis*) were reported in the northern part of the Netherlands, and later other species of waterfowl and birds of prey were also found dead. A selected number of dead wild birds were submitted for AI diagnostics and tested positive for HPAI H5N1 virus. We performed whole-genome sequencing of the HPAI H5N1 viruses found in wild birds and the 2 foxes as previously described (3) and conducted genetic and phylogenetic analyses to study the relationship between these viruses. Phylogenetic analysis of the gene segments (Appendix 1 Figure 1–8) showed the viruses detected in wild birds and the 2 foxes were in the same cluster and highly related. We classified the viruses as H5 clade 2.3.4.4b viruses, which were related to other HPAI H5N1 viruses detected in wild birds and poultry in Europe during 2020–2021. The HPAI H5N1 viruses detected in the foxes were not related to zoonotic H5N1 strains infecting humans in Asia and did not contain any known zoonotic mutations (data not shown). The sequences of the viruses detected in cub 1 (GISAID [<https://www.gisaid.org>] accession no. EPI\_2194218) and cub 2 (GISAID accession no. EPI\_2194219) were identical; the closest related virus was identified in a white-tailed eagle (*Haliaeetus albicilla*) near the village of Noordlaren. We observed only 6 aa differences: mutations A152T and T521I in polymerase basic



**Figure.** Salivating red fox (*Vulpes vulpes*) cub 1 during a fit, the Netherlands, 2021. Seizure started with retracting lips at 0 sec (A), followed by facial wrinkling with opening of mouth at 0.07 sec (B), closing of the jaws at 0.17 sec (C), then back to "normal" at 0.40 sec (D), before this sequence starts all over at 0.50 sec.

**Table.** Results of diagnostic tests of avian influenza viruses detected in 2 red fox (*Vulpes vulpes*) cubs, the Netherlands, 2021\*

Red fox	Pooled samples of brain	M-PCR1 C <sub>t</sub> value	M-PCR2 C <sub>t</sub> value	H5-PCR C <sub>t</sub> value
Cub 1	Ammon's horn and medulla oblongata	21.27	21.46	23.35
Cub 1	Cerebellum and cerebrum	19.31	20.79	21.65
Cub 2	Ammon's horn and medulla oblongata	25.72	25.92	26.44
Cub 2	Cerebellum and cerebrum	20.09	21.58	23.47

\*C<sub>t</sub>, cycle threshold; H5-PCR, subtype-specific PCR on the H5 gene; M-PCR1, PCR on the matrix gene of influenza A, repetition 1; M-PCR2, PCR on the matrix gene of influenza A, repetition 2

protein 2 (PB2); M644V in polymerase basic protein 1; A336T in nucleoprotein; L22S in neuraminidase protein; and D209N in nonstructural protein (Appendix 1 Figure 1–8). Whether these changes are associated with adaptation of the avian virus to mammal species remains unknown.

These 2 cases of infection with H5N1 clade 2.3.4.4b virus in wild red fox cubs underscore the need to raise awareness that HPAI viruses are not only zoonotic but also infect other mammal species. HPAI infection should be on the list of differential diagnoses for animals that have signs of respiratory or neurologic disease. The detection of virus in the brain suggests systemic infection of the cubs. The clinical signs were largely consistent with those reported in other natural infections of carnivores with HPAI H5 subtypes (4–7). Whether the fox cubs were infected through the parents or by eating infected bird carcasses is unclear (cubs start eating solid food at 4 weeks of age). Carnivores are known to be at risk for avian influenza virus infection upon ingesting infected birds (4,5,8). We did not test for virus shedding in these cubs, but virus shedding has been observed in experimental infection of 6- to 10-month-old red foxes with HPAI H5N1 clade 2.2 virus (8).

The United Kingdom reported infection of a red fox and seals in an animal shelter with a related HPAI H5N8 clade 2.3.4.4b virus (9), and Russia reported infection in poultry workers (10). These findings suggest that HPAI H5 clade 2.3.4.4b viruses may sporadically transmit from birds to mammals, including humans. Virus evolution and adaptive mutations must be closely monitored to rapidly identify viruses with increased zoonotic potential.

### Acknowledgments

We acknowledge the authors and submitting laboratories of the sequences from the GISAID EpiFlu Database (Appendix 2, <https://wwwnc.cdc.gov/EID/article/27/11/21-1281-App2.xlsx>).

This work was funded by the Dutch Ministry of Agriculture, Nature, and Food Quality (project no. WOT-01-003-012).

### About the Author

Dr. Rijks is a postdoctoral researcher at the Dutch Wildlife Health Centre in Utrecht, the Netherlands. Her primary research interests are wildlife diseases and epidemiology.

### References

- Van der Poel WH, Van der Heide R, Verstraten ER, Takumi K, Lina PH, Kramps JA. European bat lyssaviruses, the Netherlands. *Emerg Infect Dis.* 2005;11:1854–9. <https://doi.org/10.3201/eid1112.041200>
- Beerens N, Heutink R, Bergervoet SA, Harders F, Bossers A, Koch G. Multiple reassorted viruses as cause of highly pathogenic avian influenza A(H5N8) virus epidemic, the Netherlands, 2016. *Emerg Infect Dis.* 2017;23:1974–81. <https://doi.org/10.3201/eid2312.171062>
- Beerens N, Heutink R, Pritz-Verschuren S, Germeraad EA, Bergervoet SA, Harders F, et al. Genetic relationship between poultry and wild bird viruses during the highly pathogenic avian influenza H5N6 epidemic in the Netherlands, 2017–2018. *Transbound Emerg Dis.* 2019;66:1370–8. <https://doi.org/10.1111/tbed.13169>
- Marschall J, Hartmann K. Avian influenza A H5N1 infections in cats. *J Feline Med Surg.* 2008;10:359–65. <https://doi.org/10.1016/j.jfms.2008.03.005>
- Songserm T, Amongsin A, Jam-on R, Sae-Heng N, Pariyothorn N, Payungporn S, et al. Fatal avian influenza A H5N1 in a dog. *Emerg Infect Dis.* 2006;12:1744–7. <https://doi.org/10.3201/eid1211.060542>
- Lee K, Lee EK, Lee H, Heo GB, Lee YN, Jung JY, et al. Highly pathogenic avian influenza A(H5N6) in domestic cats, South Korea. *Emerg Infect Dis.* 2018;24:2343–7. <https://doi.org/10.3201/eid2412.180290>
- Hu T, Zhao H, Zhang Y, Zhang W, Kong Q, Zhang Z, et al. Fatal influenza A (H5N1) virus infection in zoo-housed tigers in Yunnan Province, China. *Sci Rep.* 2016;6:25845. <https://doi.org/10.1038/srep25845>
- Reperant LA, van Amerongen G, van de Bildt MW, Rimmelzwaan GF, Dobson AP, Osterhaus AD, et al. Highly pathogenic avian influenza virus (H5N1) infection in red foxes fed infected bird carcasses. *Emerg Infect Dis.* 2008;14:1835–41. <https://doi.org/10.3201/eid1412.080470>
- ProMED. Avian influenza (45): Europe (UK, Croatia) seal, fox, wild bird, HPAI H5N8. ProMED 2021 Mar 17. <http://www.promedmail.org/>, archive no. 20210317.8252821.
- ProMED. Avian influenza, human (02): Russia, H5N8, first case. ProMED 2021 Feb 21. <http://www.promedmail.org/>, archive no. 20210221.8204014.

Address for correspondence: Julianne Rijks, Dutch Wildlife Health Centre, Utrecht University, Androclus kamer O.177, Yalealaan 1, 3584CL Utrecht, the Netherlands; email: j.m.rijks@uu.nl

# Highly Pathogenic Avian Influenza A(H5N1) Virus in Wild Red Foxes, the Netherlands, 2021

## Appendix 1

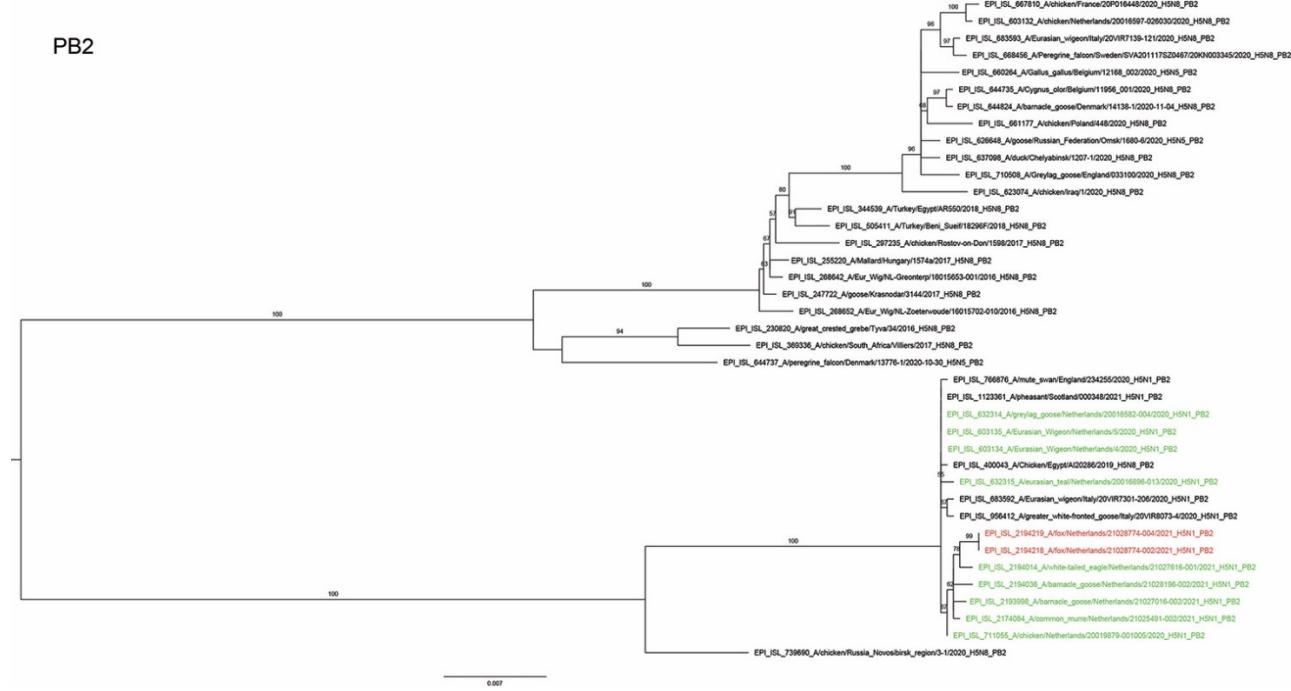
### Methods

Phylogenetic analysis was performed for each gene segment as previously described (1). Selected related sequences were obtained from GISAID's EpiFlu database on May 26, 2021 (<http://www.gisaid.org>) (2). Sequences were aligned using MAFFT v7.427 (3). Maximum likelihood trees based on the general time reversible model with a gamma-distributed variation of rates and 1000 bootstraps were generated using RAxML v8.2.12 (4) and visualized using FigTree 1.4.4 (<https://github.com/rambaut/figtree/releases>). GISAID accession numbers of the sequences and bootstrap values above 50 are shown in the phylogenetic trees. H5N1 virus sequences originating from the fox cubs are marked in red, high pathogenic avian H5N1 virus sequences from the Netherlands (2020–2021) are marked in green.

### References

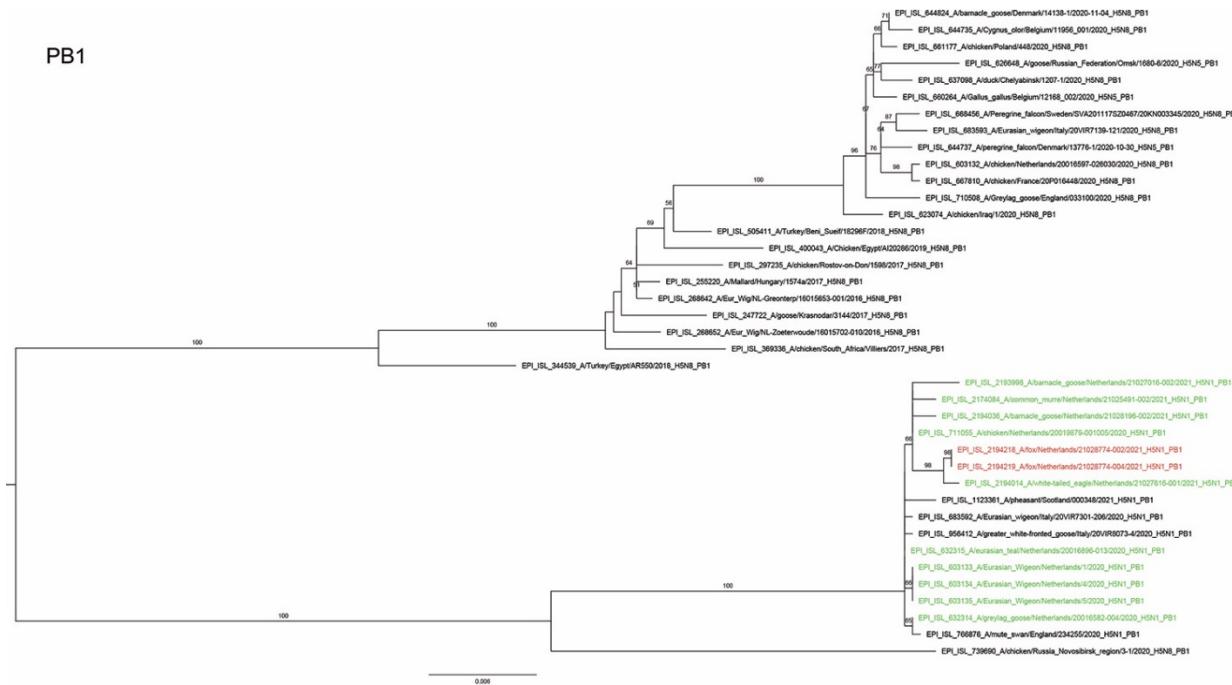
1. Beerens N, Heutink R, Harders F, Roose M, Pritz-Verschuren SBE, Germeraad EA, et al. Incursion of Novel Highly Pathogenic Avian Influenza A(H5N8) Virus, the Netherlands, October 2020. Emerg Infect Dis. 2021;27:1750–3. [PubMed](#) <https://doi.org/10.3201/eid2706.204464>
2. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro Surveill. 2017;22:30494. [PubMed](#) <https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494>
3. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. [PubMed](#) <https://doi.org/10.1093/molbev/mst010>

4. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. [PubMed](#)  
<https://doi.org/10.1093/bioinformatics/btu033>



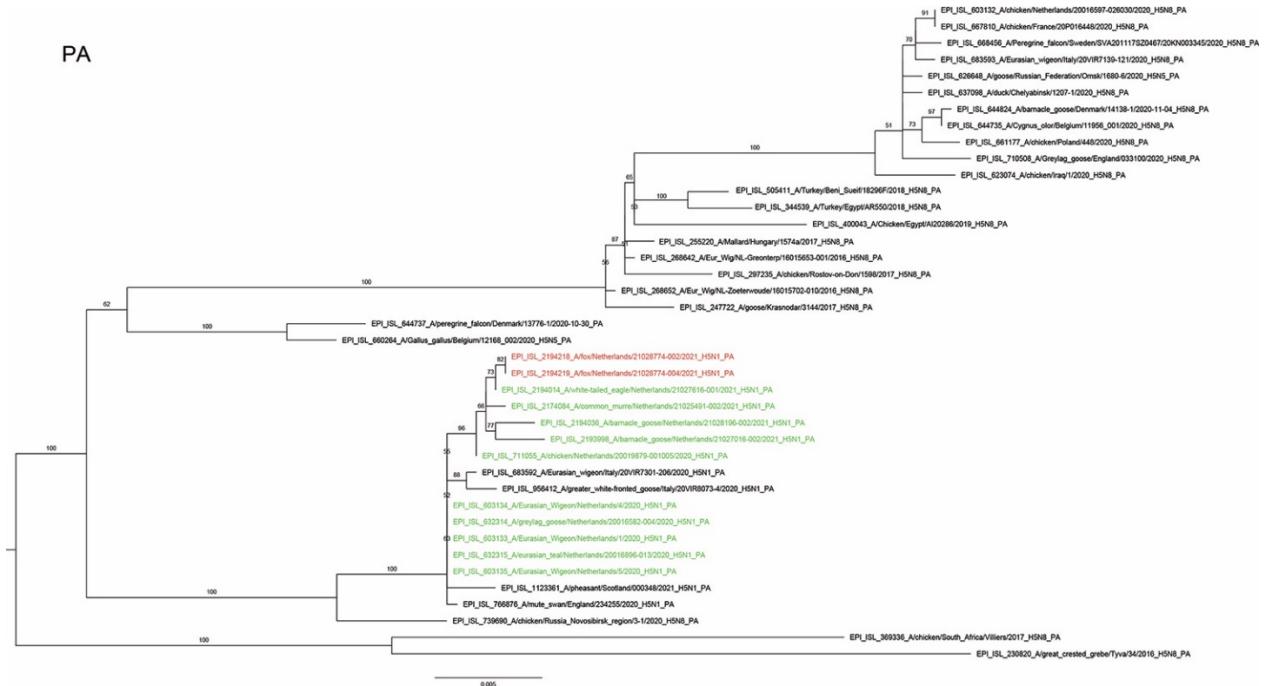
**Appendix 1 Figure 1.** Phylogenetic tree of PB2 gene segment.

PB1

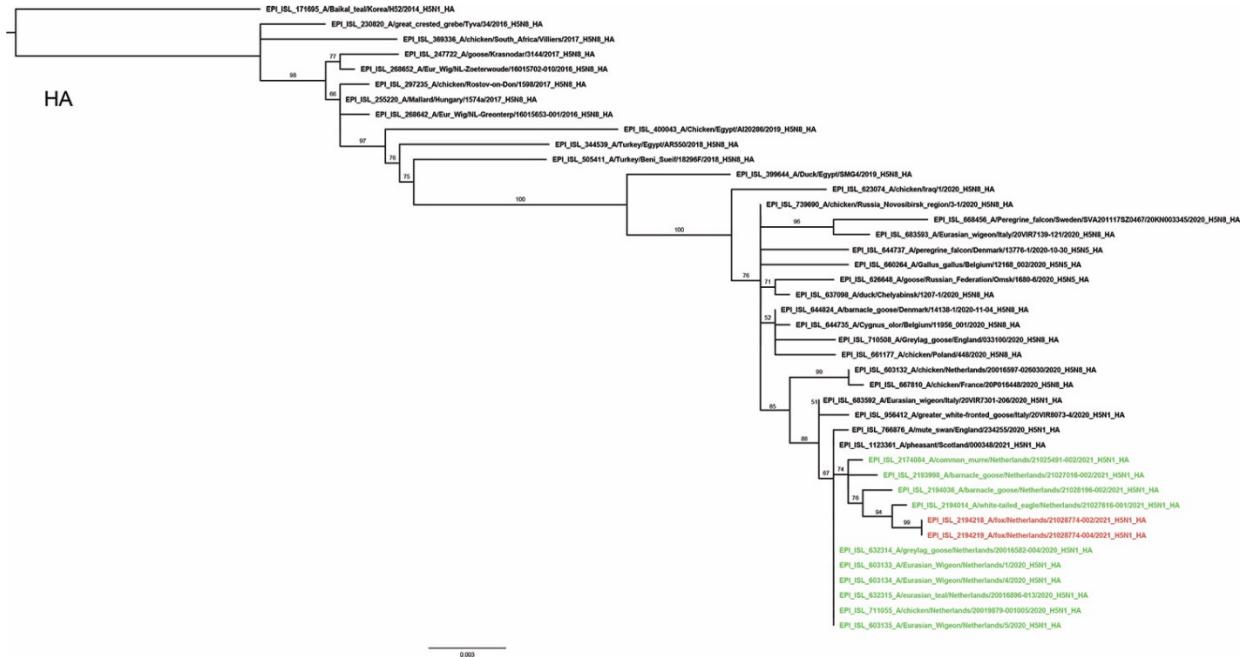


**Appendix 1 Figure 2. Phylogenetic tree of PB1 gene segment.**

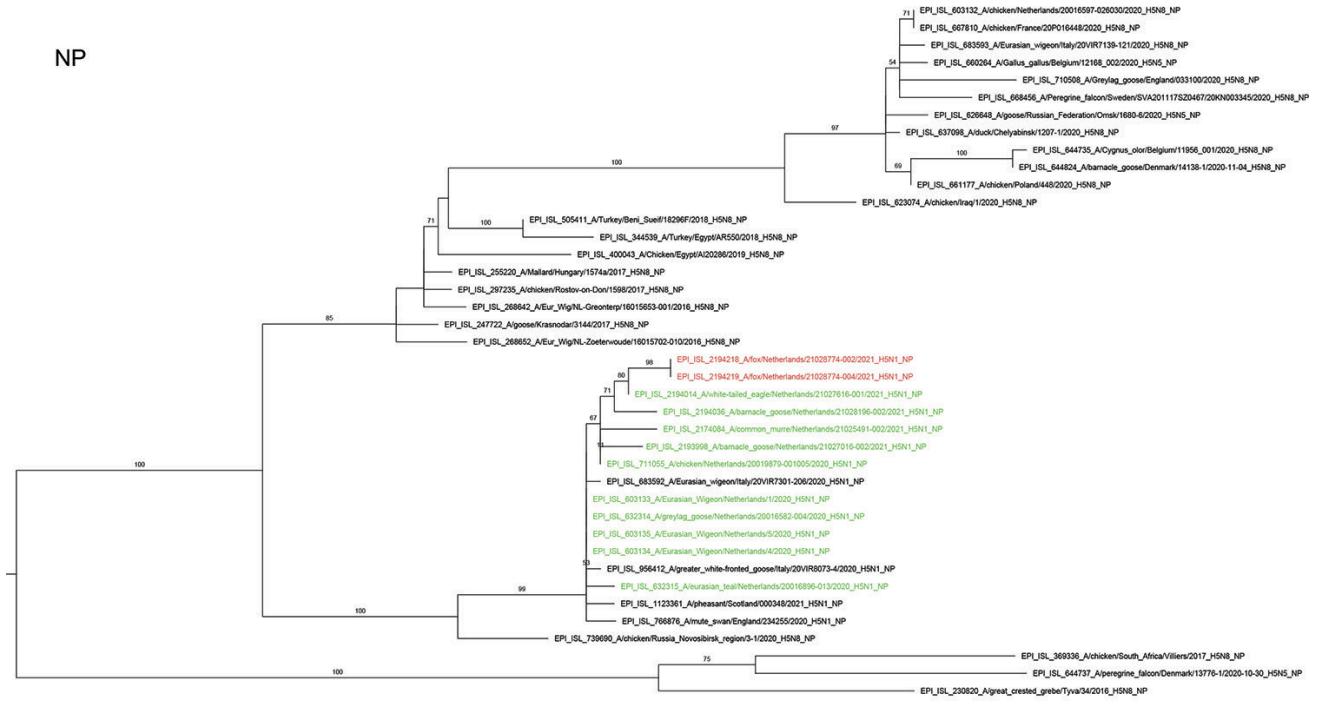
PA



**Appendix 1 Figure 3. Phylogenetic tree of PA gene segment.**

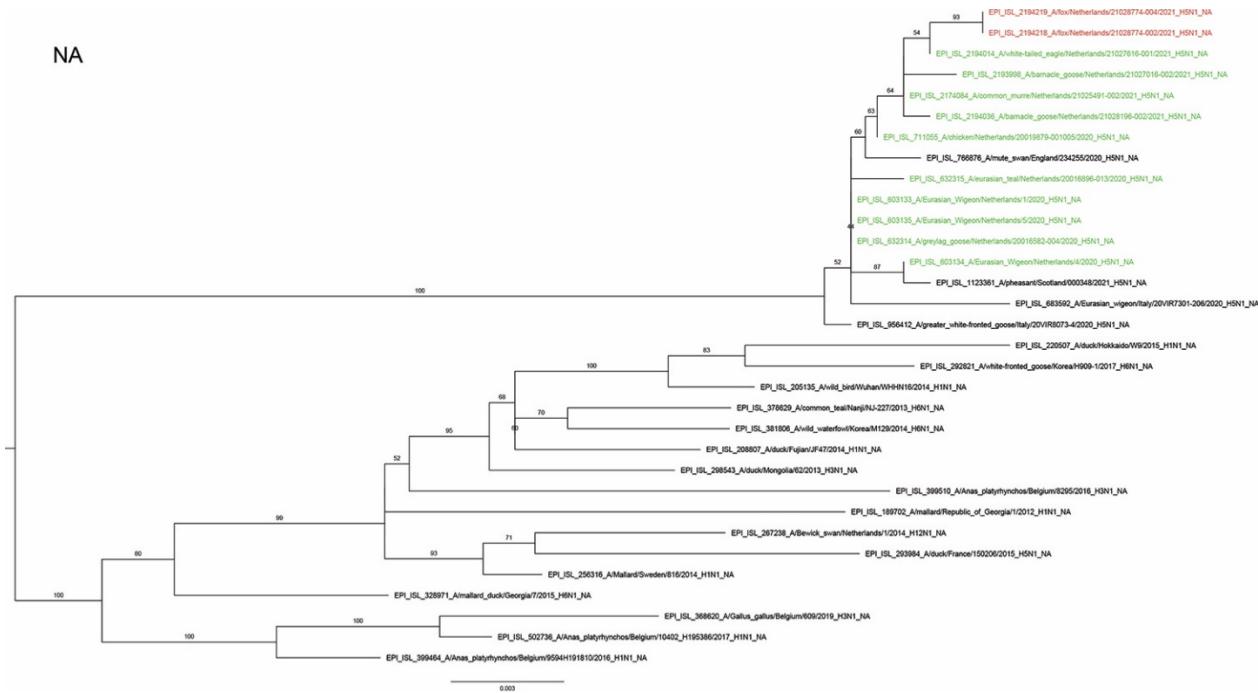


**Appendix 1 Figure 4. Phylogenetic tree of HA gene segment.**



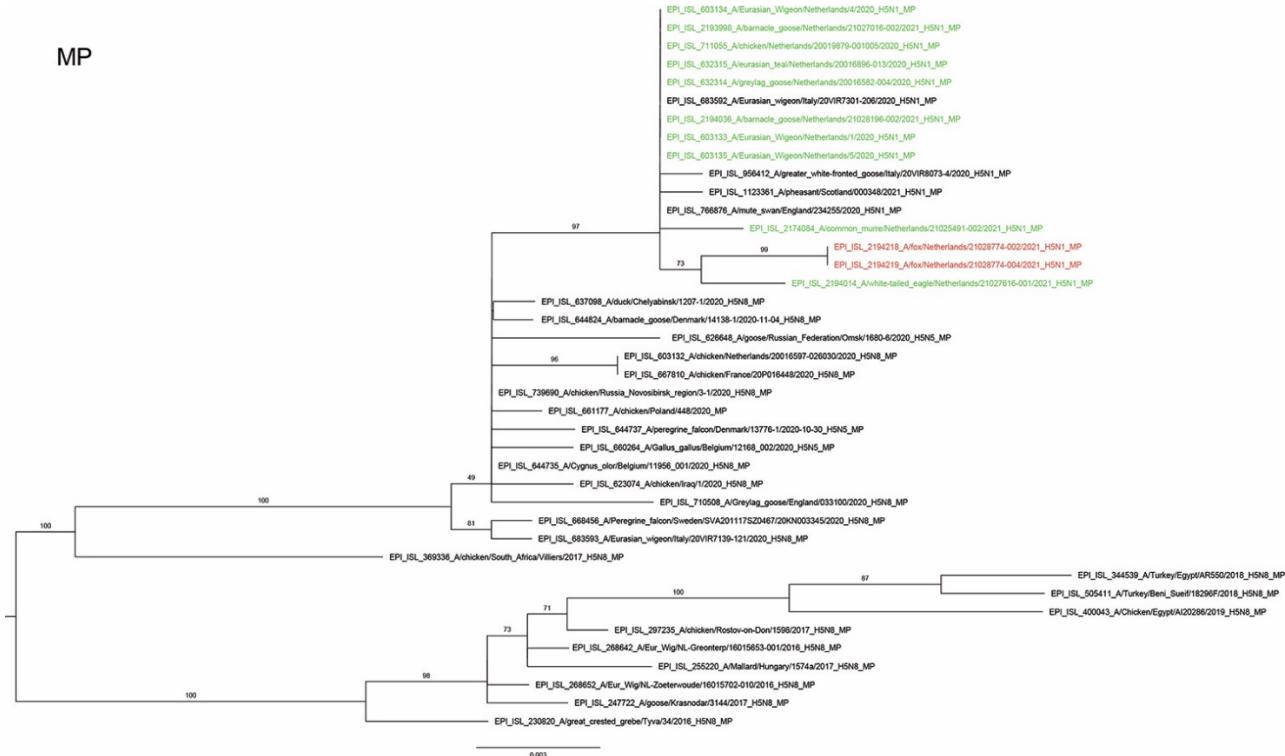
**Appendix 1 Figure 5. Phylogenetic tree of NP gene segment.**

NA



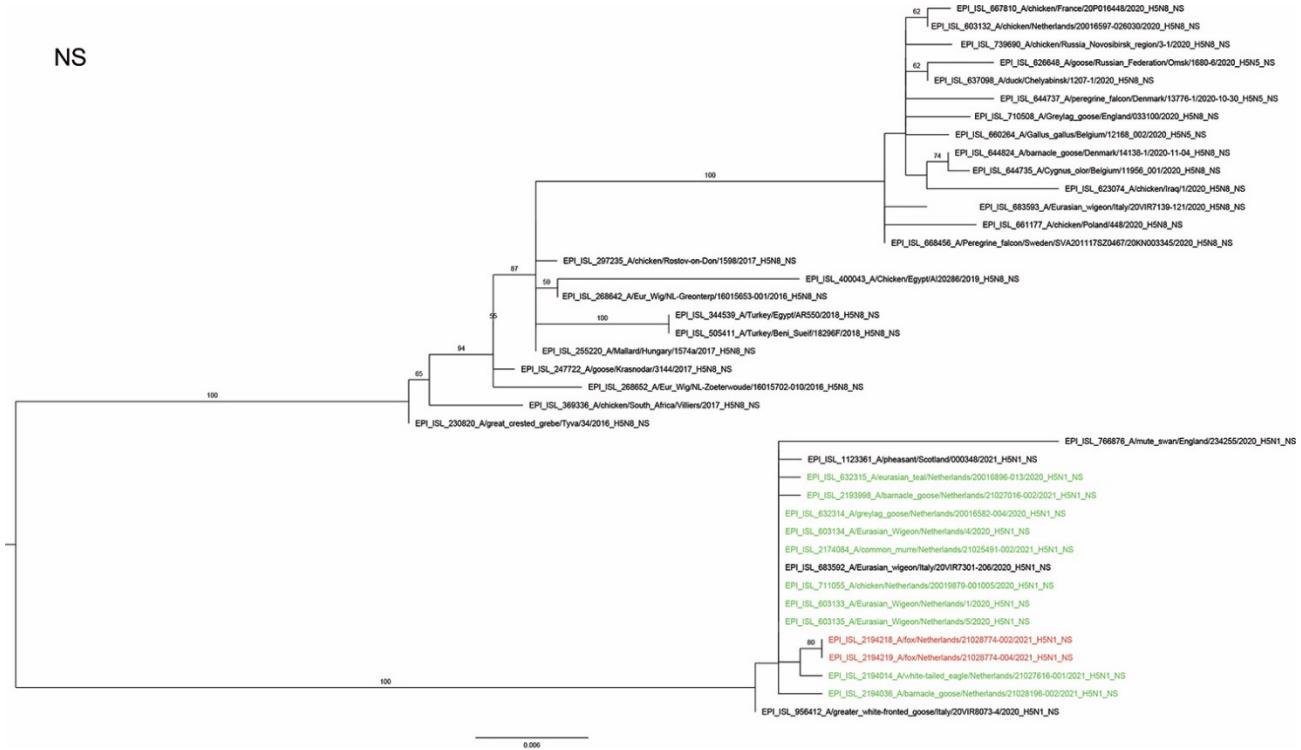
**Appendix 1 Figure 6. Phylogenetic tree of NA gene segment.**

MP



**Appendix 1 Figure 7. Phylogenetic tree of MP gene segment.**

NS



**Appendix 1 Figure 8.** Phylogenetic tree of NS gene segment.